CLAIMS

1. The use of the assessment of the binding between

- antibodies elicited against a first glycoprotein, and
- at least one glycoform of a second glycoprotein, said second glycoprotein being itself a glycoform of the first protein,

wherein said glycoform of the second glycoprotein is selected from a group of glycoforms of the second glycoprotein, each glycoform of said group corresponding to a determined glycosylation state defined by a determined sialylation state, and/or a determined branching state, and/or a determined fucosylation state, provided that said glycosylation state is not uniquely defined by a substantially unsialylated state,

for the screening of glycoform specific antibodies directed against a given glycoform of the second glycoprotein.

2. A process for screening glycoform specific antibodies among antibodies elicited against a first glycoprotein, comprising a step of determination of the binding between

- antibodies elicited against a first glycoprotein, and
- at least one glycoform of a second glycoprotein, said second glycoprotein being itself a glycoform of the first protein,

wherein said glycoform of the second glycoprotein is selected from a group of glycoforms of the second glycoprotein, each glycoform of said group corresponding to a determined glycosylation state defined by a determined sialylation state, and/or a determined branching state, and/or a determined fucosylation state, provided that said glycosylation state is not uniquely defined by a substantially unsialylated state,

to recover antibodies liable to bind to at least one given glycoform of the second glycoprotein.

3. A process according to claim 2, wherein the glycosylation state of the glycoform of the second glycoprotein presents at least one of the following criteria:

- it is essentially more sialylated than said second glycoprotein, or
- it is essentially less sialylated than said second glycoprotein, or
- it is essentially more branched than said second glycoprotein, or
- it is essentially less branched than said second glycoprotein, or
- it is essentially more fucosylated than said second glycoprotein, or
- it is essentially less fucosylated than said second glycoprotein.

4. A process according to claim 2 or 3, wherein the binding between at least one of the antibodies elicited against the first glycoprotein and each of the glycoforms of the second glycoprotein which are respectively:

- essentially more sialylated than said second glycoprotein,
- essentially less sialylated than said second glycoprotein,
- essentially more branched than said second glycoprotein,
- essentially less branched than said second glycoprotein,
- essentially more fucosylated than said second glycoprotein, and
- essentially less fucosylated than said second glycoprotein,

is determined.

5. A process according to claim 2 or 3, wherein the glycosylation state of the glycoform of the second glycoprotein presents at least two of the following criteria:

- it is essentially more sialylated or less sialylated than said second glycoprotein,
- it is essentially more branched or less branched than said second glycoprotein,
- it is essentially more fucosylated or less fucosylated than said second glycoprotein.

6. A process according to claim 5, wherein the glycosylation state of the glycoform of the second glycoprotein presents one of the following criteria:

- it is essentially more sialylated and more fucosylated than said second glycoprotein, or
- it is essentially more sialylated and less fucosylated than said second glycoprotein, or
- it is essentially more sialylated and more branched than said second glycoprotein, or
- it is essentially more sialylated and less branched than said second glycoprotein, or
- it is essentially less sialylated and more fucosylated than said second glycoprotein, or
- it is essentially less sialylated and less fucosylated than said second glycoprotein, or
- it is essentially less sialylated and more branched than said second glycoprotein, or
- it is essentially less sialylated and less branched than said second glycoprotein, or
- it is essentially more branched and more fucosylated than said second glycoprotein, or
- it is essentially more branched and less fucosylated than said second glycoprotein, or
- it is essentially less branched and more fucosylated than said second glycoprotein, or
- it is essentially less branched and less fucosylated than said second glycoprotein.

7. A process according to claim 2, 3 or 5, wherein the glycosylation state of the glycoform of the second glycoprotein presents three of the following criteria:

- it is essentially more sialylated or less sialylated than said second glycoprotein,
- it is essentially more branched or less branched than said second glycoprotein,
- it is essentially more fucosylated or less fucosylated than said second glycoprotein.

8. A process according to claim 7, wherein the glycosylation state of the glycoform of the second glycoprotein presents one of the following criteria:

- it is essentially more sialylated, more branched and more fucosylated than said second glycoprotein,
- it is essentially more sialylated, more branched and less fucosylated than said second glycoprotein,
- it is essentially more sialylated, less branched and more fucosylated than said second glycoprotein,
- it is essentially more sialylated, less branched and less fucosylated than said second glycoprotein,
- it is essentially less sialylated, more branched and more fucosylated than said second glycoprotein,
- it is essentially less sialylated, more branched and less fucosylated than said second glycoprotein,
- it is essentially less sialylated, less branched and more fucosylated than said second glycoprotein,
- it is essentially less sialylated, less branched and less fucosylated than said second glycoprotein.

9. A process according to any of claims 2 to 8, wherein the antibodies elicited against the first glycoprotein bind to the second glycoprotein with an affinity equal to or higher than the binding affinity of said antibodies to the first glycoprotein.

10. A process according to any of claims 2 to 9, wherein at least one lectin fractionation of the second glycoprotein is performed to obtain a glycoform of the second glycoprotein of a determined glycosylation state.

11. A process according to claim 10, wherein the lectin is selected from the group comprising mannose-specific lectins, such as the ConA or Lentil lectins, fucose-specific lectins, such as the Ulex lectin, gactose-specific lectins, such as ricin, or sialic acid-specific lectins, such as the limulin or Sambucus nigra lectin.

12. A process according to any of claims 2 to 11, wherein at least one enzymatic modification of the second glycoprotein is performed to obtain a glycoform of the second glycoprotein of a determined glycosylation state.

13. A process according to claim 12, wherein the enzymatic modification is carried out by an enzyme selected from the group comprising a glycosidase, in particular a neuraminidase or a fucosidase, or a glycosyltransferase, in particular a sialyl transferase or a fucosyl transferase.

14. A process according to any of claims 2 to 13, wherein a glycoform of the second glycoprotein of a determined glycosylation state is obtained by a combination of at least one enzymatic modification of the second glycoprotein and/or of at least one lectin fractionation.

15. A process according to any of claims 2 to 14, wherein a less sialylated glycoform of the second glycoprotein as compared to the second glycoprotein is obtained by neuraminidase treatment of said second glycoprotein.

16. A process according to any of claims 2 to 15, wherein a more sialylated glycoform of the second glycoprotein as compared to the second glycoprotein is obtained by sialytransferase treatment of said second glycoprotein or by neuraminidase treatment followed by sialyltransferase treatment of said second glycoprotein.

17. A process according to any of claims 13 to 16, wherein the sialyltransferase is a α-2,6 sialyltransferase, in particular a ST6GalI sialyltransferase, more particularly a N-terminal shortened ST6GalI sialyltransferase deleted of at most its first 99 residues, such as represented by SEQ ID NO: 1.

18. A process according to any of claims 2 to 17, wherein a less fucosylated glycoform of the second glycoprotein as compared to the second glycoprotein is obtained by lentil fractionation of the second glycoprotein by collecting the fraction which does not bind to lentil and a more

fucosylated glycoform of the second glycoprotein as compared to the second glycoprotein is obtained by collecting the fraction which binds to lentil.

19. A process according to any of claims 2 to 18, wherein a ConA fractionation of the second glycoprotein is performed by collecting three fractions, A, B, and C, the binding of which to ConA is such that,

- C binds to ConA more strongly than B does, and
- B binds to ConA more strongly than A does,

the branching state of a given fraction being essentially different from the branching state of the other two fractions.

20. A process according to any of claims 2 to 19, wherein, in a preliminary step, the antibodies to be screened are classified in pools, each pool being characterized in that two antibodies selected from a same pool can not bind to the same glycoprotein at the same time.

21. A process according to any of claims 2 to 20, wherein in a first step, said first step preceding the preliminary step of claim 20, it is checked that the antibodies elicited against the first glycoprotein bind to the second glycoprotein.

22. A process according to any of claims 2 to 21, wherein the binding of the antibodies to the first glycoprotein, to the second glycoprotein and to the glycoforms of the second glycoproteins is determined by using immunoassays, in particular immunoassay formats using an amplification system for detection, such as an ELISA.

23. A process according to claim 22, wherein the immunoassay is a sandwich immunoassay, in particular a sandwich ELISA test, comprising the following steps:

- fixing a capture antibody, selected from a pool such as defined in claim 20, onto a support,
- contacting a glycoprotein, corresponding to the first glycoprotein, to the second glycoprotein or to the glycoforms of the second glycoprotein, to said capture antibody, to form, if adequate, a capture antibody-glycoprotein binary complex,
- contacting a tracer antibody, selected from a pool such as defined in claim 20, provided said pool is different from the one used for the selection of said capture antibody, to said capture antibody-glycoprotein binary complex, to form, if adequate, a capture antibody-glycoprotein-tracer antibody ternary complex,

- detecting the tracer antibody for measuring the number of ternary complexes.

24. A process according to any of claims 2 to 23, wherein the first glycoprotein and the second glycoprotein are similar.

25. A process according to any of claims 2 to 23, wherein the first glycoprotein and the second glycoprotein originate from different natural tissues and/or fluids.

26. A process according to any of claims 2 to 23, wherein the first glycoprotein originates from a natural tissue and the second glycoprotein is a recombinant protein.

27. A process according to any of claims 2 to 26, wherein the first glycoprotein is a N-linked glycoprotein, such as TSH, in particular pituitary TSH, LH, FSH, or placental hCG.

28. A process according to any claim 26 or 27, wherein the first glycoprotein is pituitary or blood human TSH and the second glycoprotein is a recombinant human TSH, in particular a recombinant human TSH produced by mammalian cells.

29. The use of a glycosylation-specific antibody as screened by the process according to any of claims 2 to 28, for the binding or the purification of given glycoforms of the second glycoprotein.

30. A process for the preparation of a glycoform of a recombinant human TSH produced by mammalian cells, characterized in that said recombinant human TSH is sialylated by a α-2,6 sialyltransferase, in particular a human ST6GalI sialyltransferase, more particularly a N-terminal shortened human ST6GalI sialyltransferase deleted of at most its first 99 residues, such as represented by SEQ ID NO: 1, to yield an oversialylated glycoform of the recombinant TSH bearing α2,3 and α2,6 sialyl moieties

31. A process according to claim 30, wherein the recombinant human TSH is first treated by a neuraminidase, in particular a *Clostridium perfringens* or a *Vibrio cholerae* neuraminidase, to give a substantially unsialylated TSH, and then submitted to sialylation, to yield a resialylated glycoform of the recombinant TSH bearing essentially only α2,6 sialyl moieties.

32. A process for the preparation of a glycoform of a recombinant human TSH produced by mammalian cells, characterized in that said recombinant human TSH is submitted to a lentil fractionation, to give a lentil unbound fraction and a lentil bound fraction, the lentil unbound fraction being retained to yield a substantially unfucosylated glycoform of the recombinant TSH and the lentil bound fraction being retained to yield a glycoform which is substantially more fucosylated than said recombinant TSH.

33. A process according to claim 32, wherein:
- the recombinant human TSH is submitted to neuraminidase treatment, in particular a *Clostridium perfringens* or a *Vibrio cholerae* neuraminidase, prior to lentil fractionation, or
- the lentil bound fraction or the lentil unbound fraction of the recombinant human TSH is submitted to neuraminidase treatment,

to yield a substantially unsialylated substantially unfucosylated glycoform of the recombinant human TSH or a glycoform of the recombinant human TSH which is substantially unsialylated and substantially more fucosylated than said recombinant human TSH.

34. A process according to any of claims 30 to 32, wherein
- the recombinant human TSH is submitted to sialylation to give an oversialylated glycoform of the recombinant human TSH, or both to neuraminidase treatment and to sialylation to give a resialylated glycoform of the recombinant human TSH, prior to lentil fractionation of said glycoform, or
- the lentil unbound fraction or the lentil bound fraction of the recombinant human TSH is submitted to sialylation, or sequentially to both neuraminidase treatment and sialylation,

to yield a substantially unfucosylated oversialylated or resialylated glycoform of the recombinant human TSH or a glycoform of the recombinant human TSH which is oversialylated or resialylated and substantially more fucosylated than said recombinant human TSH.

35. A glycoform of recombinant human TSH such as obtainable according to any of claims 30 to 34.

36. A glycoform of a recombinant human TSH produced by mammalian cells which comprises from about 70% to about 100% α2,3 and α2,6 sialyl groups, in particular from

about 70% to about 85 % α2,3 sialyl groups and from about 15% to about 30% α2,6 sialyl groups.

37. A glycoform of a recombinant human TSH produced by mammalian cells which comprises from about 70% to about 100 % α2,6 sialyl groups.

38. A glycoform of a recombinant human TSH produced by mammalian cells which comprises essentially no fucose.

39. A glycoform of a recombinant human TSH produced by mammalian cells which comprises from about 30% to about 100% fucose.

40. A glycoform of a recombinant human TSH produced by mammalian cells according to claim 38, which comprises essentially no fucose and no sialyl groups.

41. A glycoform of a recombinant human TSH produced by mammalian cells according to claim 39, which comprises essentially no sialyl groups and from about 30% to about 100% fucose.

42. A glycoform of a recombinant human TSH produced by mammalian cells according to claims 36 and 38, which comprises from about 70% to about 100% α2,3 sialyl and α2,6 sialyl groups, in particular from about 70% to about 85 % α2,3 sialyl groups and from about 15% to about 30% α2,6 sialyl groups, and essentially no fucose.

43. A glycoform of a recombinant human TSH produced by mammalian cells according to claims 37 and 38, which comprises from about 70% to about 100 % α2,6 sialyl groups and essentially no fucose.

44. A glycoform of a recombinant human TSH produced by mammalian cells according to claims 36 and 39, which comprises from about 70% to about 100% α2,3 sialyl and α2,6 sialyl groups, in particular from about 70% to about 85 % α2,3 sialyl groups and from about 15% to about 30% α2,6 sialyl groups and from about 30% to about 100% fucose.

45. A glycoform of a recombinant human TSH produced by mammalian cells according to claims 37 and 39, which comprises from about 70% to about 100 % α2,6 sialyl groups and from about 30% to about 100% fucose

46. A kit for assaying specific glycoforms of a first glycoprotein, characterized in that it comprises at least one antibody such as screened according to the process of any of claims 2 to 28,

47. A kit according to claim 46, for assaying TSH in a biological sample, characterized in that it comprises:

- at least one capture-antibody selected from pools Ia, Ib, or III,
- at least a tracer-antibody selected from pools Ib, II, or III,

provided that the capture-antibody and the tracer-antibody do not belong to the same pool, wherein:

- pool Ia is defined as being the pool of antibodies which can not bind to TSH once antibody BC27, S04 , B1 has already been bound to it,
- pool Ib is defined as being the pool of antibodies which can not bind to TSH once antibody B2,R1 orS06 has already been bound to it,
- pool II is defined as being the pool of antibodies which can not bind to TSH once antibody OCD1 or R2 has already been bound to it,
- pool III is defined as being the pool of antibodies which can not bind to TSH once antibody B3 or S06 has already been bound to it.

48. A kit according to claim 46 or 47, characterized in that it comprises:

- a capture-antibody selected from pool Ia and a tracer antibody selected from pool Ib, or
- a capture-antibody selected from pool Ia and a tracer antibody selected from pool II, or
- a capture-antibody selected from pool Ib and a tracer antibody selected from pool II, or
- a capture-antibody selected from pool Ia and a tracer antibody selected from pool III, or
- a capture-antibody selected from pool Ib and a tracer antibody selected from pool III, or
- a capture-antibody selected from pool III and a tracer antibody selected from pool II.

49. A kit according to claim 47 or 48, characterized in that it further comprises a calibrant selected from the list comprising:
pituitary human TSH, recombinant human TSH produced by mammalian cells, a glycoform of recombinant human TSH produced by mammalian cells which substantially less sialylated than said recombinant human TSH, a glycoform of recombinant human TSH produced by mammalian cells which is substantially more sialylated and/or less fucosylated than said recombinant human TSH, and a glycoform of recombinant human TSH according to any of claims 33 to 36.

50. The use of a glycoprotein selected from the list comprising:
a glycoform of recombinant human TSH produced by mammalian cells which is substantially less sialylated than said recombinant human TSH, a glycoform of recombinant human TSH produced by mammalian cells which is substantially more sialylated and/or less fucosylated than said recombinant human TSH, and a glycoform of recombinant human TSH according to any of claims 35 to 45
for calibrating TSH immunoassays.